

Performance Monitoring Protocol (QA/QC) for the Thermo Q Exactive OrbiTrap LC/MS (ESI)

1 Scope

This document addresses the performance monitoring (QA/QC) of the Thermo Q Exactive OrbiTrap LC/MS system consisting of a Thermo Electron Q Exactive OrbiTrap Mass Spectrometer (MS), and a Liquid Chromatograph (LC). This document applies to personnel using the associated instrument(s)/equipment in the following discipline/category of testing: Explosives (chemistry) examinations performed at the Huntsville Laboratory.

2 Principle

The Q Exactive OrbiTrap system is comprised of a Waters LC and a Thermo Electron Q Exactive OrbiTrap high resolution MS. This system can be used for high resolution, accurate mass chemical analyses. The instrument is configured with an atmospheric pressure ionization (API) source that is capable of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). The instrument is primarily used in ESI mode. However, this protocol can also be used for APCI provided the method of ionization is clearly labeled in the resulting data. Definitions and guidelines for following this protocol are outlined in the "General Instrument Maintenance Protocol."

3 Equipment/Materials/Reagents

- a. Instrumentation - Thermo Electron Q Exactive OrbiTrap MS, API Source, Waters Acquity UPLC I class, and Data System with Xcalibur software (or equivalent)
- b. API Gas - Nitrogen, 99.99% (high purity or equivalent)
- c. Methanol (Optima grade or equivalent)
- d. LTQ Velos OrbiTrap ESI Positive Ion Calibration Solution (Thermo or equivalent)
- e. LTQ ESI Negative Ion Calibration Solution (Thermo or equivalent)
- f. Infusion Syringe - 10 to 500 μ L LC syringe (Hamilton or equivalent)
- g. Deionized Water, 18.2 M Ω Milli-Q (or equivalent)
- h. Acetone (HPLC grade)

- i. Volumetric glassware
- j. Ammonium Nitrate (NH_4NO_3), reagent grade
- Redacted**
- l. 3.125 mM Ammonium Nitrate Mobile Phase (250 mg to 1 Liter water)
- m. Waters Cortecs UPLC C18 1.6 μm , 2.1 mm X 50 mm (or equivalent)
- n. Waters Cortecs Guard Column (or equivalent)
- o. Autosampler vials - 2 mL vials, crimp or screw top with pre-split septa, with or without 100-500 μL inserts (Thermo or equivalent)

4 Standards and Controls

4.1 Testmix

The Testmix is used to assess daily operating performance, mass assignment, and continued integrity of the system. To prepare:

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- b. Testmix Solution - For daily use, dilute 20 μL of the 10 ppm stock solution to 1 mL with a 50:50 solution of methanol/water.

4.2 Calibration Solution

The calibration solution is used for coarse tuning and calibrating the mass spectrometer over the entire mass range. This procedure only needs to be performed when the instrument has been moved, down for a long period of time, undergone a major repair, or warranted based on system performance.

The calibration solution is purchased from Thermo Fisher Scientific or equivalent.

5 Calibration

The calibration procedure should be performed as needed (e.g. when the instrument has been moved, has been down for a long period of time, undergone a major repair, or warranted based on system performance).

- a. Load infusion syringe with the appropriate calibration solution.
- b. Connect the infusion syringe to the ESI probe assembly, and place in the syringe pump.
- c. Set the syringe pump to the correct syringe type and set the pump to an appropriate flow rate.
- d. Load the appropriate tune file.
- e. Check that the instrument is in the appropriate ion mode.
- f. Set the detector using the parameters listed in the 'Instrumental Conditions' section of this protocol.
- g. Engage the ESI probe and turn on the MS.
- h. Turn on the syringe pump and verify that the solution is flowing.
- i. Backup current calibration file.
- j. In the Tune page, open the Calibrate dialog box, choose the 'Semi-Automatic' tab and check the individual tests and then 'Start.'
- k. When the calibration is complete, it will display whether or not the calibration was successful.
 - If the procedure fails, repeat the calibration. If calibration continues to fail, contact the appropriate instrument support personnel.
 - When the procedure passes, print the report and evaluate the calibration solution spectrum using the 'Decision Criteria' section of this protocol.
- l. Repeat for opposite ion mode.

6 Sampling

Not applicable.

7 Procedures

7.1 Daily Checks

The following steps are to be performed daily. Enter the appropriate information in the QA/QC log for tracking purposes.

- a. Record the remaining disk space on the hard drive. Use Windows to verify that the hard disk has at least 100 MB of free disk space. Do not use if less than 100 MB remain.
- b. Record the line pressure of the building nitrogen supply (API gas). The regulator should read between 70 and 100psi. If it cannot maintain this pressure, contact the appropriate instrument support personnel. If the nitrogen is supplied by a gas cylinder, record the tank pressure. Change the tank if less than 250 psi remain.
- c. Check the oil level of the vacuum pump.
- d. Check the vacuum pressure under instrument status on the tune page. If a green circle with a white check mark in it is present, the system is ready.
- e. Record the Ultra High Vacuum pressure.
- f. To prime LC system:
 1. Open up the Acquity UPLC Console and select Acquity UPLC system in the menu on the left side of the screen.
 2. From the control drop down menu select system start up.
 3. Confirm for the sample manager (SM) that the strong wash, weak wash, and sample syringe are checked and 3 is entered in the cycles box.
 4. Confirm for the binary solvent manager (BSM) that all the boxes are checked and that the duration time is set to 3 minutes.
 5. Click the start button to start priming the system.
- g. Conduct a performance verification of the appropriate Testmix through the column. Evaluate the results using the 'Decision Criteria' section of this protocol. If the results are acceptable, print the TIC, RICs, and spectra for components in the Testmix.

- h. If all requirements are within specification, prepare records as outlined in the “General Instrument Maintenance Protocol.” If any requirements fail, contact the appropriate instrument support personnel.

7.2 As Needed Checks

- a. Replace the metal needle as needed.
- b. Clean or replace the heated capillary as needed.
- c. Clean the ion sweep cone (the heated interface front plate) as needed.

8 Instrumental Conditions

Refer to the “General Instrument Maintenance Protocol” for procedures on minor deviations.

8.1 Testmix

Liquid Chromatograph

Mobile Phase:	60% Methanol: 40% 3.125 mM Ammonium Nitrate in Deionized Water
Flow Rate:	0.5 mL/min
Column:	Waters Cortecs UPLC C18 1.6 µm, 2.1 mm X 50 mm
Inj Volume:	8 µL

Mass Spectrometer

Ionization:	ESI
Tune File:	exp_tune
Sheath Gas Flow:	20 (arb)
Aux Gas Flow:	5 (arb)
Sweep Gas Flow:	0 (arb)
Scan Mode:	Full Scan
Scan Range:	200-400 m/z (minimum)
Resolution:	17500

8.2 Calibration

Mass Spectrometer

Ionization:	ESI
Scan Mode:	Full Scan
Scan Range:	100-2000 m/z

9 Decision Criteria

9.1 Testmix

When using the OrbiTrap analyzer for accurate mass analysis, the Testmix components should be observed within the range **Reda** from their expected monoisotopic masses:

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9.2 Calibration (Positive Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Caffeine	195 m/z
MRFA	524 m/z
Ultramark	1022 m/z
	1122 m/z
	1222 m/z
	1322 m/z
	1422 m/z
	1522 m/z
	1622 m/z
	1722 m/z
	1822 m/z
	1922 m/z

9.3 Calibration (Negative Mode)

Verify the results of the calibration. The calibration will indicate if the procedure was successful. For reference, the individual ions for the calibration solution are:

Sodium dodecyl sulfate	265 m/z
Sodium taurocholate	517 m/z
Ultramark	1280 m/z
	1380 m/z
	1480 m/z
	1580 m/z
	1680 m/z
	1780 m/z

10 Calculations

Not applicable.

11 Measurement Uncertainty

Not applicable.

12 Limitations

Only properly trained personnel will perform duties involved in the operation, maintenance, or troubleshooting of this instrument.

13 Safety

Take standard precautions for the handling of all chemicals, reagents, and standards. Refer to the *FBI Laboratory Safety Manual* for the proper handling and disposal of all chemicals. Personal protective equipment should be used when handling any chemical and when performing any type of analysis. Many instrument components are held at temperatures of 250°C and higher. Precautions should be taken to prevent the contact of skin with heated surfaces and areas.

14 References

Manufacturer's Instrument Manuals for the specific models and accessories used.

"General Instrument Maintenance Protocol" (IOG 001) *Instrument Operations Group SOP Manual*.

"Liquid Chromatograph General Maintenance Protocol" (IOG 003) *Instrument Operations Group SOP Manual*.

"Mass Spectrometer General Maintenance Protocol" (IOG 004) *Instrument Operations Group SOP Manual*.

FBI Laboratory Safety Manual.

Rev. #	Issue Date	History
0	10/04/18	New document which specifics procedures for the Huntsville Laboratory.

Approval

Redacted - Signatures on File

Scientific Analysis
Unit Chief

Date: 10/03/2018

TL Approval

Explosives (Chemistry)
Technical Leader

Date: 10/03/2018

QA Approval

Quality Manager

Date: 10/03/2018